DECLARATION UNDER 37 C.F.R §1.132

I, Brian R. Genge, declare as follows:

I am a U.S. citizen, and I presently reside at 2814 Montgomery St., Columbia, SC 29205. I am an inventor of the subject matter of U.S. Patent Application Serial No. 09/978,601, the patentability of which is presently at issue. I am presently employed by the University of South Carolina, and my position is Research Associate Professor, Department of Chemistry and Biochemistry. I hold a B.S. degree in chemistry from the University of Wisconsin, and a Ph.D. in biochemistry from the University of South Carolina. I have been active in the field of biomaterials and biochemical research for the past ten years and I am the author of 40 publications covering various aspects of that field.

The information provided below describes laboratory tests that were carried out in my laboratory at the University of South Carolina to determine the compressive strength of a cement material that was produced according to the methods taught in U.S. Patent 6,013,591 to Ying, *et al.*, issued January 11, 2000.

The purpose of the tests was to enable a direct comparison between the compressive strength of a calcium phosphate cement made according to the methods taught in the Ying *et al.* patent and a cement made by the method taught in my pending patent application. The objective of the tests was to show that cements made with the calcium phosphate particles taught by Ying *et al.* provided compressive strength that was lower than the compressive strength required in certain claims of our present patent application.

Synthesis of nanocrystalline hydroxyapatite material described in U.S. Patent No. 6,013,591 by Ying *et al.*, and testing the compressive strength of a cement made from the material.

For the synthesis of the nanocrystalline apatite material, I chose to follow the "Trial 9" which Ying refers to one of the optimal methods for preparation of their material. Ying *et al.* describe the conditions at which one could expect to obtain the best results from its method as follows. At column 32, lines 56-58, of the Ying *et al.* patent, it is stated that: "[b]y using the recently optimized method for the synthesis of nanocrystalline hydroxyapatite (Trial 9 or 15), an improved nanocomposite with an even

higher compressive strength, a lower sintering temperature and greater thermal stability may be produced." At column 16, lines 61 – 63, Ying *et al.* also state: "[t]hus, the optimal calcination temperature for the pure nano-hydroxyapatite powder is 550° C degrees Celsius." And, at column 20, lines 3 – 7, it is stated that: "[s]ince room temperature processing readily yields high green and sintered densities, 25° C is the preferred reaction and aging temperature for the chemical precipitation of hydroxyapatite." Our procedure for synthesizing the nanocrystalline apatite material, therefore, was modeled according to the conditions that Ying *et al.* considered to provide the best product.

Synthesis of nanocrystalline apatite according to Ying Example 1, Trial 9:

Reagent grade $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ were used as starting materials. Aqueous solutions of $(NH_4)_2HPO_4$ and $Ca(NO_3)_2 \cdot 4H_2O$ were prepared such that the Ca:P ratio was 10:6. 0.300 M $(NH_4)_2HPO_4$ (39.6 grams per liter) and 0.500 M $Ca(NO_3)_2 \cdot 4H_2O$ (118 grams per liter) were prepared. These solutions were mixed with a magnetic stirrer. The pH of the $(NH_4)_2HPO_4$ aqueous solution was varied by adding 10 ml of concentrated NH_4OH. 300 ml of a 0.500 M solution of $Ca(NO_3)_2 \cdot 4H_2O$ was added to 300 ml of 0.300 M aqueous $(NH_4)_2HPO_4$ at a rate or 15 ml/min. The combined solution was magnetically stirred for 12 hours and aged at room temperature (25° C). The white precipitate was collected by centrifugation at 1500 rpm for 15 minutes in a Eppendorf Centrifuge 5416 swinging bucket rotor. After decanting, the precipitate was redispersed in 600 ml of a distilled water and NH_4OH solution by magnetically stirring for 20 minutes; this procedure was repeated two more times with decreasing amounts of NH_4OH and a fourth and final time with ethanol. The gel was then finely ground with an agate mortar and pestle. The gel was wet ground using a Retsch Mixer/Mill until the gel became a fine powder. The ground powders were then heat treated in air at 550° C in a Thermolyne furnace with a heating rate of 10° C./min, and a dwell time of 2 hours.

The powders thus obtained were heat treated at 550° C in air. The calcined material was then finely ground in a Retsch Mixer/Mill and the powders were uniaxially pressed in stainless steel dies at 300 MPa. The compressed material was sintered in air atmosphere by pressureless sintering in a Thermolyne furnace. Pressureless

sintering was done at 1100° C for 2 hours with a heating rate of 5° C/min. The yield was 12.09 gm of material (nanocrystalline hydroxyapatite).

Our first attempt to form a cementous material from only nanocrystalline hydroxyapatite prepared by the method of Ying *et al.*, as described above.

6.6 gms comprising solely the nanocrystalline hydroxyapatite material prepared according to Ying as described above was ground to a fine powder in Brinkmann 2000 mixer mill for 90 minutes. 3.1 gm of the ground powder was then further mixed manually in a chilled agate mortar and pestle with 0.80 ml of 0.60 M Na_2HPO_4 containing 1.5% wt/vol sodium polyacrylicate, 60,000 average molecular weight, and mixed for 2 minutes until it formed a paste. The paste was placed into Delrin molds to form round 9.8 mm diameter by 6.8 mm height cylinders. After 10 minutes at 37° C, the cylinders were removed from the molds and further aged in 100% humidity atmosphere at 37° C. After 24 hours the compressive strength of the cylinders was tested using a Shimadzu Autograph AGSH-1 compression tester. The compressive strength of the cylinders (n = 3) was 4.65 ± 1.98 MPa.

Our second attempt to form a cementous material from nanocrystalline hydroxyapatite prepared by the method of Ying *et al.*, as described above.

4.6 gms of the nanocrystalline hydroxyapatite material prepared according to Ying *et al.* as described above, was combined with 0.5 gm $CaCO_3$ and 0.69 gm $Ca(H_2PO_4)_2 \cdot H_2O$ and ground to a fine powder in Brinkmann 2000 mixer mill for 90 minutes. 3.1 gm of the ground powder was then further mixed manually in a chilled agate mortar and pestle with 0.80 ml of 0.60 M Na_2HPO_4 containing 1.5% wt/vol sodium polyacrylicate, 60,000 average molecular weight, and mixed for 2 minutes until it formed a paste. The paste was placed into Delrin molds to form round 9.8 mm diameter by 6.8 mm height cylinders. After 10 minutes at 37° C, the cylinders were removed from the molds and further aged in 100% humidity atmosphere at 37° C. After 24 hours the compressive strength of the cylinders was tested using a Shimadzu Autograph AGSH-1 compression tester. The compressive strength of the cylinders (n = 3) was 25.28 ± 0.46 MPa.

Comparison with the compressive strength of cement made by the method described in our present patent application:

In Examples 1 - 5 of our present patent application, we show how to make a biocompatible cement that cures quickly and rapidly develops a high compressive strength. In Figure 3, in fact, the compressive strength of our material is shown to be about 50 MPa at the end of one hour, and to develop a compressive strength of over 90 MPa after 11 hours. The compressive strength at 24 hours would be expected to be even higher. By way of comparison, cements made according to the methods described by Ying *et al.* were measured to have compressive strengths that ranged from about 2.5 MPa to about 26 MPa (4.65 ± 1.98 MPa and 25.28 ± 0.46 MPa)

Conclusion and analysis:

In conclusion, it is evident that cement made by the methods described in U.S. Patent No. 6,013,591 to Ying *et al.*, have much lower compressive strength than a cement that is made according to the methods that we disclose in our application, and, in fact, never develop a compressive strength that is more than one-half of the 65 MPa that is described in the claims.

In my opinion, the compressive strength that was demonstrated by the cement in our second attempt to form cement with the Ying *et al.* apatite, although quite low, was due primarily to the reaction between the commercially available $CaCO_3$ and $Ca(H_2PO_4)_2 \cdot H_2O$, which were components in the cement mixture, rather than the presence of the nanocrystalline hydroxyapatite of Ying *et al.* One reason for this is that crystalline hydroxyapatite is the most thermodynamically stable forms of calcium phosphate, and the very low solubility of sintered hydroxapatite (Ksp = 2.9 X 10-42 (from Fulmer, M. T. *et al., Biomaterials, 23(3)*:751 – 755 (2002)), prevent it from being useful as a biological cement since the dissolution rate of the calcium and phosphate ions is too slow to allow it to undergo the dissolution-reprecipitation process involved in calcium phosphate cementous reactions. Accordingly, and as what would have been expected by one skilled in the art, it is not surprising that the highly crystalline material of Ying *et al.* failed to form a cement having a high compressive strength, as does the highly reactive, substantially amorphous TCP nanoparticles of the present invention.

I declare that all statements herein made of my own knowledge are true and that all statements made herein on information and belief are believed to be true. I do hereby state that I am aware that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. §1001) and may jeopardize the validity of the application or any patent issuing thereon.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Executed on: 12-18-03 (Date)

Signature: Brian Genge.

Brian R. Genge